Rejection of Claims 1-23 Under 35 U.S.C. § 112, First Paragraph

Claims 1-23 have been rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement. The Examiner states that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with the claims. In particular, the Examiner indicated that the specification is enabling for a method of transducing explanted and perfused hearts of C57BL/6 mice with 1.5 x 10.9 IU of rAAV/CMV-lacZ for 15 minutes via a catheter in the left carotid artery. However, the Examiner appears to believe that this does not reasonably enable a method of treating a cardiovascular condition by infusing rAAV into a coronary artery or a coronary sinus for a period of time in an amount sufficient to stably and efficiently transduce cardiomyocytes perfused by the artery or sinus, wherein the rAAV vector encodes at least one nucleic acid operably linked to a control region, wherein the nucleic acid encodes a therapeutically effective molecule, and expressing the therapeutic molecule in an amount effective to ameliorate the cardiovascular condition in a mammal.

Applicants respectfully traverse the rejection. The invention of claims 1-23 is directed to a method of treating a cardiovascular condition comprising infusing a recombinant adeno-associated virus (rAAV) vector into a coronary artery or coronary sinus for a time and in an amount sufficient to stably and efficiently transduce cardiomyocytes perfused by the artery or the sinus, wherein the vector comprises at least one nucleic acid encoding a therapeutically-effective molecule, which is operably linked to a control region, and expressing the molecule in an amount effective to treat or ameliorate the cardiovascular condition.

The Examiner indicates that the state of the art regarding cardiovascular gene therapy at the time of filing would have been considered unpredictable for one skilled in the art to treat or ameliorate any cardiovascular condition in any mammalian species. In particular, the Examiner states that it is not readily apparent how the expression of lacZ gene is correlative of or broadly enables the expression of any therapeutic gene. Moreover, the Examiner asserts that the specification is not enabling for failing to teach a correlation between the expression of lacZ and the expression of a therapeutic transgene encoded by rAAV to treat any cardiovascular or ameliorate any cardiovascular condition in any mammalian species.

However, the specification provides sufficient information to enable one having ordinary skill in the art at the time the claimed invention was made to make and use the invention without undue experimentation. As acknowledged by the Examiner, the *in vitro* and *in vivo* examples at pages 10-12 demonstrate the delivery and transduction of cardiomyocytes using the method of the claimed invention with an rAAV vector encoding the gene for β-galactosidase. The vector was delivered in PBS to adult mouse heart *ex vivo* by perfusion into the carotid artery. The perfused hearts were then transplanted into hosts. Applicants observed high levels of transduction particularly after 4 and 8 weeks after transplant. At 2, 4, and 8 weeks, the level of cardiomyocytes observed expressing β-gal was <1%, about 40%, and >50%, respectively. Thus, Applicants have clearly demonstrated stable and efficient transduction of transgenes to cardiomyocytes according to the claimed delivery method.

Applicants submit that once rAAV-mediated delivery of a transgene to cardiomyocytes was discovered by Applicant in accordance with the present invention, one having skill in the art at the time the claimed invention was made would have known that delivery of a transgene to cardiomyocytes would evoke a sufficient biological response therapeutically-effective to treat a

cardiovascular condition targeted by the selected transgene. For example, the reference, R.G. Crystal, Science, 270:404-410 (1995) (hereinafter "Crystal") provided by the Examiner in the Office Action discloses that genetically transferred material evokes a biologic response relevant to the underlying disease in several studies. Crystal at 407. In particular, Crystal discloses the treatment of ADA deficiency by delivery of normal ADA cDNA, the treatment of familial hypercholesterolemia by delivering LDL receptor genes, and the treatment of cystic fibrosis by delivery of the CFTR gene. *Id.* Moreover, Crystal describes one such study recognizing that when adenovirus vectors are administered, the animals developed circulating neutralizing antibodies directed against the vector. *Id.* at 408. Thus, one having skill in the art would have expected that delivery of the appropriate therapeutic transgene to cardiomyocytes as taught by Applicants would evoke a biologic response to treat the contemplated cardiovascular condition.

This conclusion is further manifested by the fact that delivery and expression of genes by the rAAV vector to skeletal muscle cells have been previously described. Podsakoff *et al.*, U.S. Patent No. 5,858,351, and Kessler *et al.*, "Gene Delivery To Skeletal Muscle Results In Sustained Expression And Systemic Delivery Of A Therapeutic Protein," *Proc. Natl. Acad. Sci. USA*, 93:14082-87 (Nov. 1996)(hereinafter "Kessler"), attached hereto, describe the delivery of genes such as erythropoietin and β-galactosidase to skeletal muscle cells. Kessler specifically provides that the rAAV vectors efficiently transduce skeletal muscle, resulting in long-term stable protein expression. Kessler at 14085. The authors state that the feasibility of gene therapy using the rAAV vector "is supported by studies showing that gene delivery to skeletal muscle [], vascular smooth muscle [], and liver [] can result in systemic levels of therapeutic proteins." *Id.* at 14082. Thus, Podsakoff and Kessler demonstrate that at the time the claimed invention was made, once a method of delivering the therapeutic gene to the skeletal muscle of interest was

developed, one having skill in the art could reasonably expect expression of a therapeutic gene to evoke the desired biologic response. Therefore, the therapeutic treatment of cardiovascular conditions by the claimed method was enabled at the time the claimed invention was made.

There have been reported specific examples of treating cardiac conditions by delivering a transgene encoding a therapeutically-effective protein to evoke a therapeutically-beneficial response and thereby treat the cardiac condition. For example, the Hammond patents, U.S. Patent Nos. 5,792,453 and 6,100,242, attached hereto, describe methods of using different transgenes encoding angiogenic proteins or peptides to stimulate coronary collateral vessel development in patients, or increasing contractile function in the heart of a patient. Specifically, Hammond describes the transfer of genes encoding angiogenic proteins such as aFGF, bFGF, and FGF-5 using an adenovirus expression vector and demonstrates collateral blood vessel formation. U.S. Patent No. 6,100,242, col. 6, lines 1-19. Furthermore, Leiden, U.S. Patent No. 5,661,133, attached hereto, specifically describes expressing a protein in cardiac myocytes by injecting a plasmid expression vector encoding a protein that induces angiogenesis into the myocardium of a mammalian host and demonstrates the production of collateral blood vessel formation. U.S. Patent No. 5,661,133, col. 6, lines 22-25 and 43-50 and Fig. 7. Induction of angiogenesis is known for treatment of ischemic cardiac conditions. Thus, the high level of skill in the art in combination with the specification provides adequate teaching to make and use the claimed invention with a reasonable expectation of success by providing the skilled artisan with a delivery method that permits stable and efficient transduction of cardiomyocytes by rAAV encoding a transgene of interest for treatment of a cardiovascular condition of interest.

The Examiner cites Crystal and W.F. Anderson, *Nature*, 392:25-30 (1998)(hereinafter "Anderson") for the proposition that experimental studies in mice are not predictive of safety and

efficacy in humans. However, the Crystal and Anderson references do nothing to discourage one skilled in the art from developing new gene therapy methods. Rather, they provide a summary of the groundwork already laid by many scientists in the field that would lead one to a conclusion opposite of the Examiner's, *i.e.*, that there is a promising future in gene therapy. While Crystal and Anderson note obstacles from prior work, this does not lead to a conclusion that gene therapy is altogether unsafe and non-therapeutic, as alleged. To the contrary, these references note the important contributions and successes already found with gene therapy protocols.

Crystal establish that human gene transfer is feasible and successful. Crystal at 405, third column. Specifically, Crystal states that "most studies have shown that genes can be transferred to humans whether the strategy is *ex vivo* or *in vivo*, and that all vector types function as intended." *Id.* Moreover, Table 1 provides a summary of studies specifically demonstrating the feasibility of gene transfer. Furthermore, Crystal explicitly addresses the administration of the adenovirus vector for *in vivo* studies. In this context, Crystal notes that an adenovirus vector containing the normal human cystic fibrosis transmembrane conductance regulator cDNA was delivered and transferred to the nasal or bronchial epithelium of individuals with cystic fibrosis. *Id.* at 406. Moreover, Anderson specifically provides that adenovirus-associated viral vectors appear to be safe and can efficiently transduce muscle cells. Anderson at 28. Accordingly, Crystal and Anderson provide evidence of the suitability, feasibility and safety of gene transfer using the rAAV vectors of the present invention in mammals.

The Examiner further states that the use of the rAAV vectors in the present invention would have been considered unpredictable. However, as the Examiner pointed out, Anderson discloses that AAV vectors are used in clinical trials as being the only known mammalian virus showing preferential integration into a specific region in the genome. The Examiner's reliance

on Anderson's comments that the rAAV vectors of the present invention appear to integrate in a non-specific manner, are limited to DNA of 4.8 kb, and that the production of viral particles is labor intensive because efficient packaging cell have yet to be developed appear misplaced. This is particularly evident because Anderson concludes that "these [rAAV] vectors hold promise and appear to be safe." Anderson at 28. Despite these asserted observations, Anderson believes that the rAAV vectors are suitable for gene therapy.

Moreover, the fact that the Examiner alleges that the invention is unpredictable completely ignores the fact that Applicants successfully obtained efficient transduction and expression of the β-gal protein in cardiomyocytes in accordance with the present invention. One of the most difficult hurdles to overcome in gene therapy is getting DNA into the a so that expression can take place. Historically, plasmid vectors are known to be the least efficient vector to insert DNA into cells. Nevertheless, despite this inefficiency, scientists have been able to get therapeutic levels of expression from DNA by transfected a plasmid into a cell vector, *e.g.*, Leiden. More recently, with the use of more efficient viral vectors, such as adenoviruses and rAAV, even more cells are transduced. With greater transduction, a greater amount of expression is found. Accordingly, once Applicants established that rAAV DNA could be inserted into cardiomyocytes and expression of a marker gene obtained therefrom by the claimed method, there is a reasonable expectation of success to select a particular gene to treat a cardiac condition of interest to achieve treatment without resort to undue experimentation.

Furthermore, Applicants respectfully believe that the specific requirements of safety and efficacy charged by the Examiner is not an issue for the Patent Office to decide, but one regulated by the Food and Drug Administration. Enablement requires that the specification provide an adequate written description to teach one how to make and use the claimed invention

without undue experimentation. Applicants have done exactly what is required under the first paragraph of 35 U.S.C. § 112. Applicants teach the skilled artisan how to make the rAAV vectors of the present invention and how to use the rAAV vectors to deliver transgenes to cardiomyocytes, which are expressed therein. Nothing in Crystal or Anderson rebut these teachings by Applicants.

As to the selection of particular elements involved in the claimed process, including time period for infusion, the particular promoter, and the specific therapeutic molecule, Applicants respectfully submit that the determination of these elements would not require undue experimentation. As set forth above, the level of skill in the art as exemplified by the Hammond patents, Leiden patent, Podsakoff patent, and Kessler reference teach that one having skill in the art would understand that the selection of such elements is within the purview of the artisan. Moreover, Applicants provide a description of suitable regulatory elements, *e.g.*, at page 6, line 25 through page 7, line 2; the time period for infusion and amounts of rAAV infused to ameliorate a cardiovascular disease, *e.g.*, at page 8, lines 27-30, and page 9, lines 1-7; and the method of delivery, *e.g.*, at page 7, lines 3-15. Therefore, contrary to the Examiner's position, Applicants clearly enable the full scope of the present invention and withdrawal of the instant rejection is respectfully requested.

Conclusion

Applicants respectfully submit that the present invention is now in condition for allowance and an indication of allowability is respectfully requested. In the event that the

Examiner believes that a personal interview would expedite prosecution, she is invited to call the undersigned.

Sincerely,

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